*Environmental and Experimental Botany*, Vol. 27, No. 2, pp. 223 to 226, 1987.
Printed in Great Britain.

0098-8472/87 $3.00 + 0.00
Pergamon Journals Ltd.

# GASEOUS COMPOUNDS OF SOYBEAN TISSUE CULTURES: CARBON DIOXIDE AND ETHYLENE EVOLUTION*

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(*Received* 26 *February* 1986; *accepted in revised form* 5 *August* 1986)

ZOBEL R. W. *Gaseous compounds of soybean tissue cultures; carbon dioxide and ethylene evolution.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **27,** 223–226, 1987.—Soybean cultivars Clark 63, Wayne and Chippewa 64 show identical $CO_2$ evolution patterns from hypocotyl segments during callus induction. Newly developing callus in sealed chambers shows the slowest rate of $CO_2$ evolution; that of aerated chambers shows the highest. Although $CO_2$ evolution is identical for the three cultivars, their growth rates are significantly different, suggesting that $CO_2$ evolution is not completely coupled to growth. Growth of hypocotyl callus in sealed chambers results in the production of a gaseous compound(s) which reduces net carbon dioxide evolution and biomass accumulation of callus tissues.

Wayne and Chippewa 64 have high rates of ethylene evolution compared to Clark 63. At 25°C Clark 63 hypocotyl tissue cultures produce little ethylene while intact seedlings are noted for higher than normal ethylene evolution from the hypocotyl. This suggests differential gene expression. Ethylene accumulations (in sealed chambers at 6 days) from both cv. Wayne and cv. Chippewa 64 are in the phytotoxicity range. This potential for phytotoxicity must be taken into account when using sealed vessels.

*Key words:* Soybean, tissue culture, gaseous atmospheres.

## INTRODUCTION

PHYTOACTIVE gases are produced by plant tissue cultures.[3,6,10] ZOBEL and ROBERTS[10] postulated that one phytoactive gas, ethylene, affects cytodifferentiation, but not cell division or callus growth. If ethylene has a suppressing effect on cytodifferentiation, its accumulation in culture vessels could inhibit differentiative phenomena such as regeneration.

Since documented levels of ethylene accumulation are frequently above minimum phytotoxic levels, the evolution and accumulation patterns of ethylene and its competitive inhibitor carbon dioxide need to be explored for all tissue culture systems where characteristics other than growth are to be studied.

Temperature induced inhibition of soybean hypocotyl elongation has been shown to be cultivar dependent.[2,5] SAMIMY and LAMOTTE[8] demonstrated that these differences were due to increased ethylene synthesis of one cultivar (cv. Clark 63) when the temperature was increased from 20°C to 25°C while other cultivars did not change their rates of ethylene synthesis. Comparisons between cultivars in tissue culture are

* Contribution of the U.S. Department of Agriculture, Agricultural Research Service, and Cornell University, Departments of Agronomy and Plant Breeding.

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infrequently made. Clark 63 (high ethylene production at 25°C) will be compared with other cultivars to document levels and the significance, if any, of endogenous ethylene *in vitro*.

## MATERIALS AND METHODS

Seeds of soybean cultivars Chippewa 64, Wayne and Clark 63 were obtained from our Long Island Field Trials, sterilized for $\frac{1}{2}$ hr with 2.625% sodium hypochlorite, rinsed twice in sterile distilled water, and placed on water saturated filter paper in Petri dishes to germinate. After 5 days seedlings were removed, hypocotyls sliced into approximately 2-mm long segments and the segments placed in Petri dishes containing modified MURASHIGE and SKOOG[7] media.[*vide* 10] Five–nine Petri dishes with nine hypocotyl segments per plate were placed in 1-l, gas tight chambers in a dark incubator at 25°C. This was chosen to provide the optimum temperature for maximum ethylene evolution from Clark 63 hypocotyl segments.[8] Ethylene determinations were made with a poropak Q, 1 m long × 3.17 mm ID copper column with air as the carrier gas, and an electrical conductivity detector. Carbon dioxide concentration was determined by thermal conductivity with a micro-bead thermistor placed in series with the ethylene detector. Sampling in all cases consisted of 1 ml samples of gases taken via a rubber septum, and injected into the gas chromatograph.

In experiment 1 chambers were kept open or closed for 6 days. The open chambers were sealed for 1 hr prior to the daily sampling, and reopened immediately after sampling. This experiment was repeated six times with two chambers per treatment.

Experiment number 2 consisted of three treatments: (1) chambers of each cultivar kept sealed and sampled daily, (2) chambers left sealed for 24 hr, after which they were sampled, opened, blown out with a stream of air for 5–10 min, and then resealed, and (3) chambers sealed and filter sterilized laboratory air circulated through at 2 l/hr and exhausted outside. The chambers from treatment 3 were sealed daily for 1 hr immediately prior to sampling. This experiment was repeated six times with two chambers per treatment.

Statistical analysis was performed using the MATMODEL program[4] to provide the $F$ test and standard deviations.

## RESULTS AND DISCUSSION

The three cultivars grew quite differently in the open chambers of experiment 1 (Fig. 1). Cv. Wayne had the highest growth rate and cv. Clark 63 the lowest. This differential growth may have conditioned some of the results of the experiments reported here. The differential growth, however, was not correlated with carbon dioxide evolution (Fig. 2). The carbon dioxide evolution patterns were virtually identical for all three cultivars. Clark 63, with the least growth, was statistically unaffected by culture conditions (Table 1), while Wayne and Chippewa 64 hypocotyl segments produced significantly less callus in the closed chambers as compared to that for the open chambers. Because of the nature of the experiments, it is assumed that a volatile compound, given off by the culture medium or the tissue itself, is inhibiting growth.

There was differential ethylene evolution by the three cultivars in the closed chambers (Fig. 3). The rate of ethylene evolution in the open chambers was too low for accurate measurement after 1 hr of accumulation, so only ethylene accumulation in the closed chambers was measured. Contrary to expectation, Clark 63 callus evolved ethylene at a significantly lower rate than Wayne or Chippewa 64. This is the reverse of what SAMIMY and LAMOTTE[8] found with intact soybean hypocotyls, suggesting that gene expression in callus is, at least in some cases, independent of the source of the callus. Recent results[1,9] suggest that the loss of effect may be due to the excision of cotyledons.

Experiment 2 confirmed and extended the results of experiment 1. The method of sampling $CO_2$ evolution has a dramatic effect on the results obtained. Figure 4 compares rates of $CO_2$ evolution amongst the three treatments for cv. Wayne (data for Chippewa 64 and Clark 63 are identical), and Table 1 presents the weights of callus at the end of the experiment. Different treatments have characteristic effects on the rate of $CO_2$ evolution. An analysis of variance of the data in Table 1 demonstrates that with Chippewa 64 and

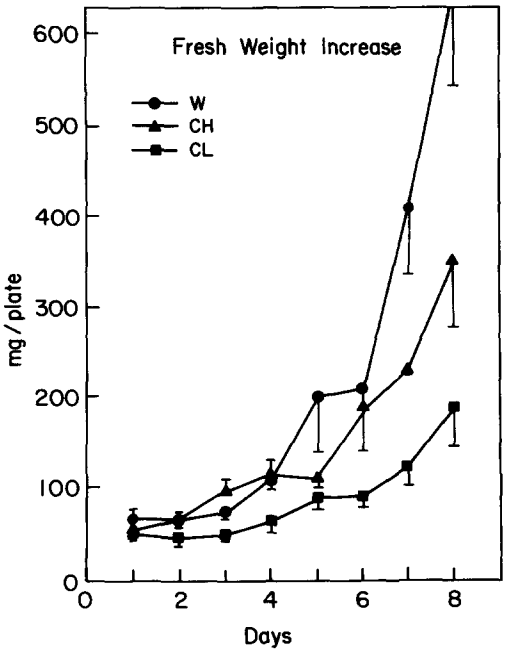


FIG. 1. Fresh weight increase for the three cultivars cv. Wayne (W), cv. Chippewa 64 (CH) and Clark 63 (CL) over an 8-day period cultured in open chambers. Error bars represent mean ± S.D.

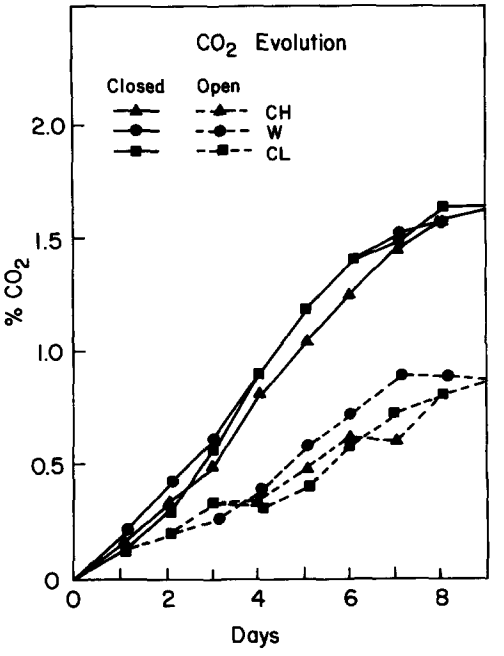


FIG. 2. Eight day $CO_2$ mean evolution curves for open and sealed chambers for cv. Wayne (W), cv. Chippewa 64 (CH) and cv. Clark 63 (CL) (statistics not presented).

Wayne there is a significant effect of different treatments on final callus weight with the air treatments intermediate between that for opened and sealed chambers. Clark 63 callus growth is not significantly different between treatments.

It is obvious that the technique used to maintain tissue cultures has an effect on their development. Sealing soybean hypocotyl segment tissue cultures in air tight or nearly air tight chambers results in a reduction in growth, or conversely, providing soybean hypocotyl segments and callus with fresh air stimulates growth. Several tentative conclusions can be developed from these results: (1) Several volatile substances (including $CO_2$ and ethylene) are produced or accumulate under air tight culture conditions. (2) A substance(s), as it accumulates, inhibits $CO_2$ evolution from tissue cultures. (3) There is no apparent relationship between $CO_2$ and ethylene evolution. (4) Growth rate and $CO_2$ evolution in soybean hypocotyl segment tissue cultures are not correlated. (5) Gene expression in tissue culture can be independent of the source of the tissue.

*Table* 1. *Weights of callus after* 8 *days of culture in sealed, opened, or continuous flow (air) chambers*

| cv | Weight (mg) | | | |
|---|---|---|---|---|
| | Air | Sealed | Opened | *F* |
| Wayne | 1104 | 582 | 921 | 4.6* |
| Chippewa 64 | 1081 | 730 | 876 | 6.9† |
| Clark 63 | 943 | 733 | 907 | 2.0 NS |

* Significant at 5% level.
† Significant at 1% level.
NS, Not significant.

Caution must be urged for those working with tissue cultures under sealed or reduced aeration conditions. Carbon dioxide, ethylene and other volatile compounds can accumulate and may

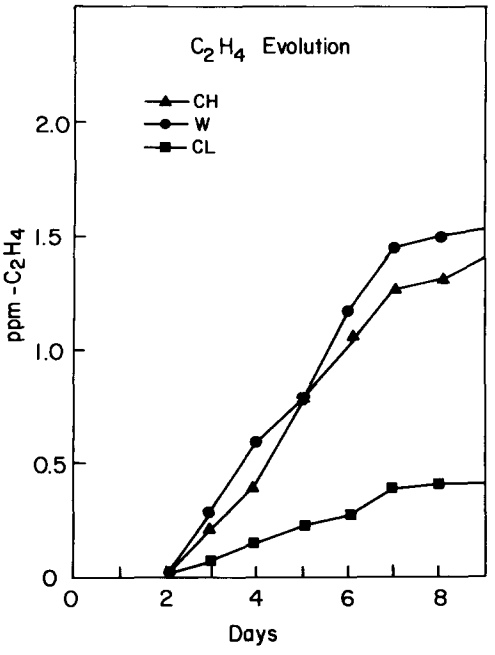


FIG. 3. Eight-day mean ethylene evolution curves for hypocotyl segment cultures from cv. Wayne (W), cv. Chippewa 64 (CH) and Clark 63 (CL), in sealed chambers (statistics not presented).

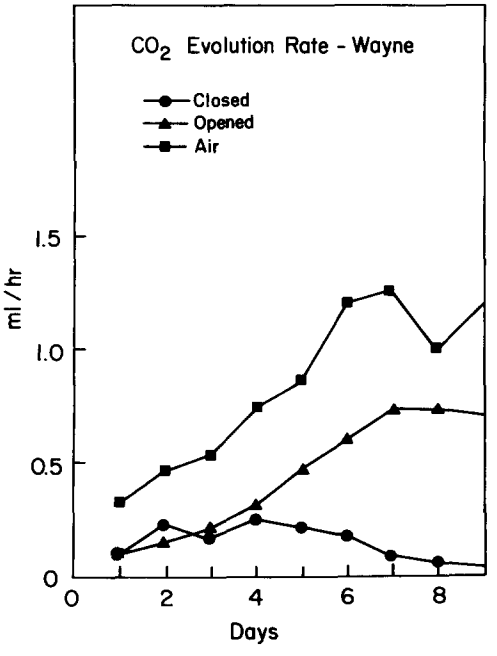


FIG. 4. Evolution rate of $CO_2$ under different cultural conditions: closed chambers, opened chambers and continuous flow air. Mean data for cv. Wayne (statistics not presented).

modify growth, differentiation, and ultimately the applicability of results.

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